

New World camelids and BVDV (Bovine Virus Diarrhea Virus) infection in Switzerland:  
a retrospective study

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Abstract

The aim of this study was the search for persistently infected (PI) New World camelids (NWC) as a possible source of Bovine Virus Diarrhea Virus (BVDV) infection for other NWC or cattle in Switzerland, where an eradication program for BVDV has been implemented. Different organs from 166 animals and 101 sera from different parts of Switzerland were tested for BVDV antigen by means of immunohistochemistry and HerdChek\*BVDV Ag/Serum Plus. None of the organs and sera was found to be positive for antigen.

Up until now, PI NWC are infected mainly by BVDV genotype 1b. In Switzerland most PI cattle harbor the subgroup BVDV-1e, followed by 1h, 1k and 1b. The subgroup BVDV-1b is found in less than 10% of the cattle cases. Therefore, assuming that NWC are more prone for a persistent infection with the subgroup 1b, it could be hypothesized that the infection rate of NWC is lower than in other countries because of a lower circulating level of this type in ruminants in Switzerland. We conclude that NWC are a negligible threat to the eradication efforts for BVD in Switzerland.

Key Words: Llama, Alpaca, Bovine virus diarrhea virus, ELISA, necropsy, immunohistochemistry

## Introduction

New World camelids (NWC) are becoming more and more popular as pets and for leisure activities like trekking. In 2009 2'652 llamas and 2'094 alpacas were registered in Swiss farms (Bundesamt für Statistik, Berne CH). A wide range of viral infections are known in NWC, like West Nile Virus, contagious ecthyma and Bovine virus diarrhea virus. Persistent BVDV (Bovine Virus Diarrhea Virus) infections have been reported in recent years in alpacas (Goyal et al., 2002; Mattson et al., 2006; Foster et al., 2005 and 2007; Carman et al., 2005; Barnett et al., 2008; Byers et al., 2009; Kim et al., 2009) and in llamas (Belknap et al., 2000; Wentz et al., 2003).

BVDV belongs to the genus pestivirus within the flaviviridae family. The genus consists of the two genetic species BVDV-1 and -2, which are again divided into numerous subgroups. Among BVD viruses, cytopathogenic (cp) and non-cytopathogenic (ncp) types are distinguished due to their properties when cultured on cells. Only the non-cytopathogenic type causes persistent infection in cattle when affecting fetuses during the immunotolerant phase of gestation (Bachofen et al., 2009; Van Amstel and Kennedy, 2010). In alpacas an ncp BVDV genotype 1b was isolated (Goyal et al., 2002; Carman et al., 2005; Byers et al., 2009; Kim et al., 2009) and in llamas also a BVD type 1b was recognized (Wentz et al., 2003). A serological survey among 63 alpaca herds all over the USA found 16 (25.4%) of them to harboring seropositive crias and 4 (6.3%) with persistent infected (PI) crias (Topliff et al., 2009). A similar survey in Switzerland including 53 alpaca and llama herds with 109 sera examined detected a seroprevalence of 4.6% (Danuser et al., 2009). A newer serological survey of 596 serum samples showed that the prevalence of BVDV carriers was 0% (Mudry et al., 2010).

Attempts were made to provoke the birth of PI crias by experimental infection of 4 pregnant llamas between days 65 and 105 of gestation. But neither were clinical signs observed in the mothers nor were PI crias born (Wentz et al., 2003). Because the

gestation period in NWC is longer than in cattle (alpacas: range 335 to 356 days; NWC mean 345 days), the period of susceptibility of the camelid fetus for a persistent infection has not been determined with certainty (Carman et al., 2005; Mattson et al. 2006; Byers et al., 2009; Kapil et al., 2009; Van Amstel and Kennedy, 2010). Supposed that the ontogenesis of the immune system is similar to bovids, Mattson et al. (2006) postulate the development of a PI cria until approximately 145 days of gestation to occur/be possible. Other authors (Byers et al., 2009) propose that the gestational exposure time for BVDV immunotolerance in alpacas may be only the first trimester. In a newer study, transplacental infection during early gestation of alpacas naturally exposed to BVDV type 1b was confirmed in 7 out 10 live-born crias (Benedice et al, 2011).

In PI crias clinical symptoms like ill thrift, anorexia, decreased weight gain, chronic recurrent debilitation and infections as well as diarrhea can be found and some show congenital defects. Stillbirths and abortions are seen too in affected herds (Evermann, 2006; Foster et al., 2007; Byers et al., 2009; Passler and Walz, 2009; Topliff et al., 2009; Van Amstel and Kennedy, 2010; Bedenice et al., 2011).

For diagnosis of BVDV in NWC the same diagnostic test designed and used for the identification of BVDV infection in cattle can be used, as immunohistochemistry, antigen detection ELISA, PCR or virus isolation (Carman et al., 2005; Kapil et al., 2009). By using immunohistochemistry large amounts of antigen can be found in PI crias in several organs (Byers et al., 2009). As source of infection in NWC movement of animals is suspected, which means, e.g. female alpacas with crias dislocating between premises for mating. Another possibility would be the contact to infected cattle, mixed animal husbandry and communal pastures (Evermann, 2006; Foster et al., 2007; Barnett et al., 2008; Danuser et al., 2009; Passler and Wetz, 2009; Topliff et al., 2009; Van Amstel and Kennedy, 2010).

Because an eradication of BVDV is in progress in Switzerland, the aim of this study was to exclude NWC as a possible source of reinfection of bovines by identifying BVDV infected animals. In this study we therefore used retrospective paraffin-embedded material available at the institute of veterinary pathology from 1996 to 2009 and serum samples from NWC collected in 2007 to identify possible infection with pestiviruses, mainly BVDV.

## Material and methods

Paraffin-embedded material from, 94 alpacas, 59 llamas, 5 guanacos and 8 vicuñas referred for necropsy to the Institute of Veterinary Pathology, between 1996 and 2009 was available.

The llamas send in for necropsy were from neonatal to adult animals, one abortion was examined and the oldest animal was 16 years old, the female to male ratio was 50:50. 74 % of alpacas in the necropsy were females. The age of the incoming animals was from neonatal to 27 years, among them 6 abortions were investigated. From the 5 guanacos 3 were females, 2 out of them neonatal and one 12 years old, one animal was a male of 3 years and one animal an abortion. Five vicuñas were female from neonatal to 23 years, one male animal was 4 days of age. Furthermore, one stillbirth and one abortion were examined. Most animals were from the German part of Switzerland (mostly from the canton Zurich, see table 1 and figure 1).

In addition to this retrospective data, 99 serum samples from sound animals collected during a study for the survey of infectious diseases (Kaufmann et al., 2010; Zanolari et al., 2010) were analyzed by ELISA for BVD antigen. Serum samples originated from 77 alpacas and 24 llamas. Age and gender distribution of this sample is presented in table 2.

## Immunohistology

Tissue sections mostly of brain but also skin, spleen, kidney, liver or gastrointestinal tract were deparaffinized and rehydrated, watered in tap water for 5 min. and counterstained for 2 min. in hemalaun. Endogenous peroxidase was inactivated by treatment with 3% H<sub>2</sub>O<sub>2</sub> (3% H<sub>2</sub>O<sub>2</sub> with 0,2% NaN<sub>3</sub> (sodium azide) in water) for 10 min. at room temperature (RT). Afterwards the slides were digested with proteinase k for 10 min. at room temperature (Dako REAL Proteinase K (40x), S2019). For 1 h at 37° C the slides were incubated with the following two antibodies: C42 (dilution 1:400; Prof. V. Moennig, Institute of Virology, Hannover, Germany) and 15c5 (dilution 1:1000; Dr. E. Dubovi, Cornell University, USA). Afterwards the EnVision-method

(DAKO, K 4001 EnVision, peroxidase mouse, Zug, Switzerland) was applied as described by the manufacturer. As chromogen AEC (Aminoethyl Carbazole Substrate Kit, 00-2007, Invitrogen) was used. As a positive control the analogous stained brain section from a PI calf was used.

#### HerdChek\*BVDV Ag/Serum Plus

This ELISA detects BVDV antigens in serum, plasma, whole blood and ear notch tissue samples. Specific monoclonal antibodies (Er<sup>ns</sup>/gp44-48) are coated on the microtiter plates, so that captured BVD-antigens can be detected (HerdChek\* BVDV Antigen ELISA Ear-Notch/Serum Test Kit; Idexx Laboratories). This ELISA is not established in NWC; however, it was used in previous studies or mentioned in reviews (Foster et al., 2005; Kapil et al., 2009; Van Amstel and Kennedy, 2010).

#### Results

##### Necropsy

In 18 out of 59 llamas send in for necropsy the main problem was a moderate to severe infestation with *Dicrocoelium dendriticum* and subsequent liver necrosis and fibrosis (cirrhosis). 7 had a severe infestation with intestinal parasites like *Eimeria* sp. or *Nematodirus* sp., 5 had a septicemia, 3 had each a lymphoma and two a carcinoma in diverse organs, 2 had a *Clostridium perfringens* enterotoxaemia (type D) and the rest of the animals had diverse problems like arthritis, cardiovascular problems, birth trauma, lung problems or no diagnosis. No abortion cause could be determined in the only abort examined.

An infestation with *Dicrocoelium dendriticum* was seen at the autopsy in 12 out of 93 alpacas, 1 animal was infested with *Fasciola hepatica*, 11 animals showed cachexia mostly because of dental problems (hooks and waves) but also because of parasitic infestation, 6 animals had intestinal parasites like trichostrongylides, from the 6 abortions two had a septicemia, one a pneumonia and the placenta a suppurative

placentitis suggesting a bacterial infection, one showed a intrauterine asphyxia and two had no diagnosis. Two neonatal alpacas died because of inanition, two because of poorly unfolded lung and two adult alpacas had a generalized mycobacterial infection, 7 out of 93 had a pneumonia or bronchopneumonia, 4 had a endocarditis valvularis, birth deformities like ventricular septum defect, choanal atresia, spina bifida occulta, hydrocephalus internus were found in single juvenile animals and the oldest animal (27 years) had bilateral ocular cataract. Other problems like septicemia (3 animals), tubulonephrosis and urolithiasis (one animal each) were also found.

The abortion and one adult animal in the guanaco group (n=5) had no diagnosis, one animal died from a cerebrocortical necrosis, one showed degeneration of muscle fibers due to vitamin E/selenium deficiency and one died because of trauma induced by a herdmate.

Lymphoma was found in an adult vicuña, one neonatal animal showed septicemia, another uremia due to a glomerulonephritis and tubulonephrosis, a 4 days old animal showed a suppurative meningitis, the stillbirth and the aborted animal had no diagnosis, one animal had arthrosis and one stomatitis papulosa.

## Immunohistology

Tissues of 166 animals were examined for BVDV antigen by means of immunohistochemistry as described above. In no animal, BVDV antigen could be detected by this test (Figure 2).

## HerdChek\*BVDV Ag/Serum Plus

Two out of the 101 sera examined were repeatedly positive. A skin biopsy of these cases was requested. For immunohistochemistry, however, only one case could be examined and revealed to be negative in IHC.

## Discussion

190 Organs from 166 animals and 101 sera were tested for BVDV antigen. Assuming that  
191 the population number of NWC in Switzerland was around 4'000 at the time of  
192 sampling (Bundesamt für Statistik, Berne, Switzerland), approximately 6.7% of this  
193 population was involved in our investigation. Danuser et al. (2009) found a  
194 seroprevalence of 4.9%; however, as a population of mainly sick animals in a referral  
195 centre was analysed there is a potential bias in this study. Mudry et al. (2010) found  
196 an overall pestivirus seroprevalence of 5.75% and a seroprevalence of 0% for BVDV  
197 in the year 2008 in NWC in Switzerland, showing that the infection rate is low and that  
198 the exposure of NWC with BVDV is a rare event. Both exposition and susceptibility  
199 can be regarded as low. Still movement of animals for mating or contact to infected  
200 cattle and mixed animal husbandry and communal pastures has to be regarded as a  
201 source of infection in NWC (Foster et al., 2007; Barnett et al., 2008; Topliff et al.,  
202 2009; Danuser et al., 2009). The review of the necropsy reports demonstrates that  
203 infestation with *Dicrocoelium dendriticum*, seldom with *Fasciola hepatica*, but with  
204 intestinal parasites too has been (Wenker et al., 1998) and still is one of the bigger  
205 problems in NWC farming.

206 Immunohistochemistry is described in the literature as a strong tool for identifying PI in  
207 NWC (Carman et al., 2005; Byers et al., 2009). With the HerdChek\*BVDV Ag/Serum  
208 Plus we had 2 positive results out of 101 sera. One of the positive sera was confirmed  
209 to be false positive by IHC. Kapil et al. (2009) described this phenomenon and they  
210 postulate that commercial antigen-capture ELISA can cause false positive results  
211 because of high background. The antigen-ELISA has not been validated for camelids  
212 (Van Amstel and Kennedy, 2010). However, the second positive serum sample could  
213 not be rechecked by IHC testing because the owner of the animal was not willing to  
214 carry out further examinations.

215 The PI NWC described in the literature up until now were infected mostly by the  
216 genotype 1b. In Switzerland the viral genetics of 169 Swiss isolates from bovines  
217 confirmed the presence of the BVDV-1 subgroups b, e, h and k. No BVDV type 2 was  
218 detected in this study (Bachofen et al., 2008). In another study in PI cattle, most  
219 animals harbored the subgroup BVDV-1e, followed by 1h, 1k and 1b. The subgroup  
220 BVDV-1b was found in less than 10% of the cases (Bachofen et al., 2009). Therefore,  
221 assuming that NWC are more prone for a persistent infection with the subgroup 1b it

can be hypothesized that the infection rate of NWC is lower than in other countries because of a lower circulating level of this type in ruminants in Switzerland.

We conclude that in Switzerland there is a low risk of an infection of NWC with the subgroup BVDV-1, mostly with the genotype 1b and that the PI in NWC crias can be regarded as a rare event. Furthermore, NWC are a negligible threat to the eradication efforts for BVD in Switzerland.

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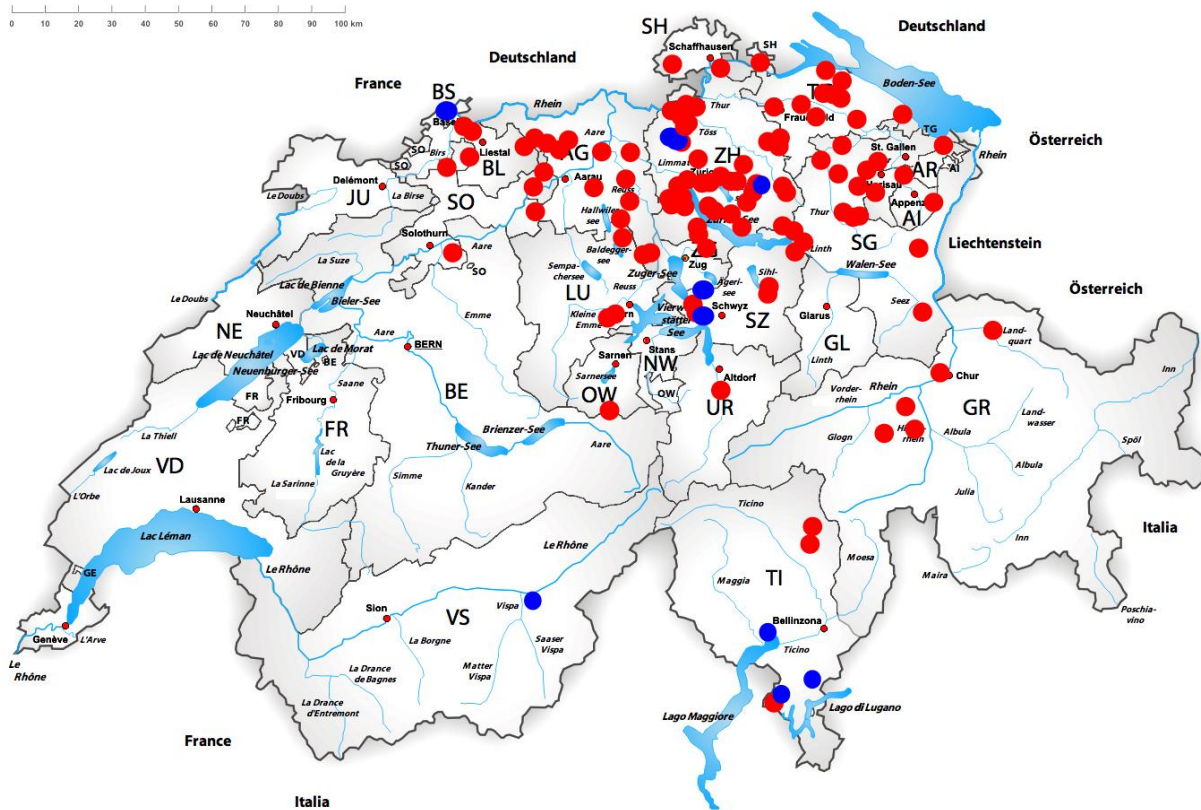
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1 Figure 1: Nachbarländer Bezeichnung in Englisch (Italia -> Italy)

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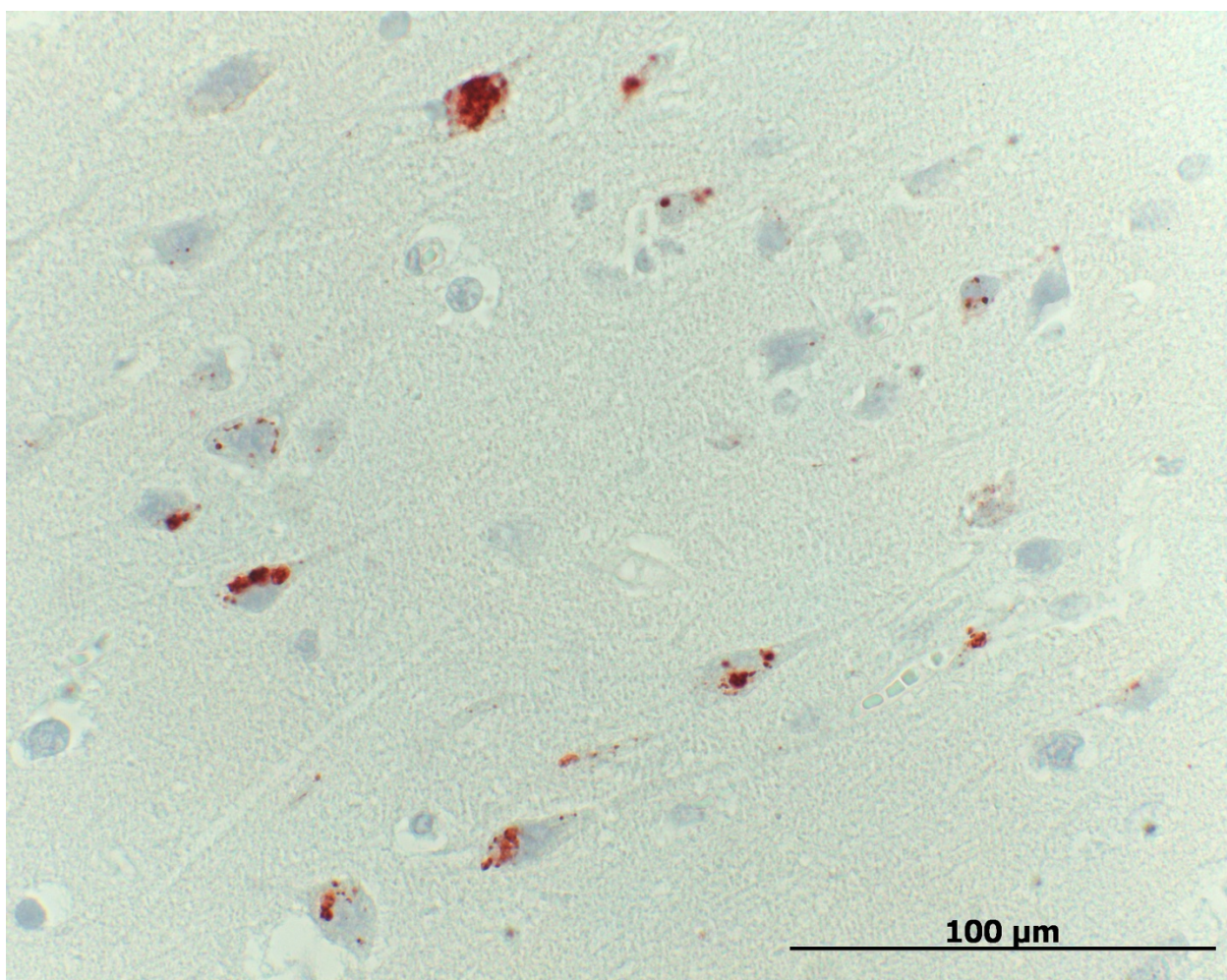
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34 Table 1:

<b>Canton</b>	<b>Male</b>	<b>Female</b>	<b>Abortion/ unknown</b>
Argovia	4/3/0/0	10/4/0/0	0/0/0/0
Inner Rhodes	0/1/0/0	0/1/0/0	0/0/0/0
Basle-Country	1/1/0/0	1/1/0/0	1/0/0/0
Grisons	0/5/0/0	1/0/0/0	1/1/0/0
Lucerne	2/2/0/0	1/1/0/0	0/0/0/0
Schaffhausen	1/1/0/0	2/2/0/0	0/0/0/0
Solothurn	1/0/0/0	2/0/0/0	0/0/0/0
Schwyz	3/1/0/0	1/1/0/0	0/0/0/0
St. Gall	4/0/0/0	7/2/0/0	2/2/0/0
Ticino	2/0/0/0	2/0/0/0	0/0/0/0
Thurgovia	5/2/0/0	6/2/0/0	0/1/0/0
Uri	1/0/0/0	0/0/0/0	0/0/0/0
Zug	0/0/0/0	2/1/0/0	0/0/0/0
Zurich	12/11/1/1	15/12/3/5	4/1/1/2

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36 Table 2:

<b>Species</b>	<b>Male</b>	<b>Female</b>	<b>Median age</b>	<b>Min. age</b>	<b>Max. age</b>
Alpaca	20	57	3.2	0.1	20
Llama	9	15	3.1	0.1	18.5

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**Mycobacterial -> welche Mycobacterien?**

Species	Number of animals	Necropsy results
<b>Llama</b>	18	Infestation with <i>Dicrocoelium dendriticum</i>
	7	Intestinal parasites, like <i>Eimeria</i> sp. and <i>Nematodirus</i> sp.
	5	Septicemia
	3	Lymphoma
	2	Carcinoma
	2	<i>Clostridium perfringens</i> enterotoxaemia (type D)
	22	Diverse problems, like arthritis, cardiovascular problems, birth trauma, lung problems or no diagnosis
<b>Alpaca</b>	12	Infestation with <i>Dicrocoelium dendriticum</i>
	1	Infestation with <i>Fasciola hepatica</i>
	11	Cachexia due to dental problems or endoparasitosis (e.g. <i>Nematodirus battus</i> )
	6	Infestation with parasites like trichostrongylides
	7	Pneumonia or bronchopneumonia
	4	Endocarditis valvularis
	4	Neonatal animals: Inanition (n = 2), poorly unfolded lungs (n = 2)
	2	Generalized mycobacterial infection (in one case <i>M. kansasii</i> was isolated and in the other no cultural identification was possible)
	6	Abortion due to septicemia (n = 2), pneumonia and suppurative placentitis (n

		= 1), intrauterine asphyxia (n = 1), no diagnosis ( n = 2)
	4	Birth deformities like ventricular septum defect, choanal atresia, spina bifida occulta and hydrocephalus internus
	3	Septicemia
	2	Cataract
	1	Tubulonephrosis
	1	Urolithiasis
<b>Guanaco</b>	1	Cerebrocortical necrosis
	1	Vitamin E/Selen deficiency
	1	Trauma
	2	No diagnosis
<b>Vicuña</b>	1	Lymphoma
	1	Septicemia
	1	Uremia
	1	Suppurative meningitis
	1	Arthrosis
	1	Stomatitis papulosa
	2	Stillbirth and abortion: no diagnosis

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Legend:

**Figure 1:** Distribution of the New World camelids (NWC) examined in this study. Blue dots are the localization of serum sampling, red dots are the localization of NWC from where animals were received for necropsy.

**Figure 2:** Immunohistochemistry. BVDV positive control, bovine brain: EnVision-method, 40x. Note the intracytoplasmic red labelling of neurons.

**Table 1:** Regional and gender distribution of cases analyzed  
(Alpaca n=94/Llama n=59/Guanaco n=5/Vicuña n=8)

**Table 2:** Serum samples for BVD antigen ELISA, age (years) and gender distribution

**Table 3:** Most common necropsy diagnoses between 1996 and 2009

Zanolari Patrik 16.4.11 12:22

**Kommentar [1]:** 10 blaue Punkte auf der Karte für 59 Lamas und 93/94 Alpacas?